CHAPTER 10

Provitamin A Carotenoids: Occurrence, Intake and Bioavailability

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10.1 Introduction

Carotenoids range, together with terpenoids, among the most prevalent, lipid-soluble secondary plant metabolites. In plants, carotenoids are often affiliated with the light-harvesting complexes, preventing the photo-oxidation of chlorophyll, and also absorbing light in the 450–500 nm region, closing the ''green-gap'' of chlorophyll absorption. In addition, they can be found in chromoplasts, adding to the typical yellow-to-red coloration of many plants.

This prevention of photo-oxidation has added to the reputation of carotenoids as antioxidants, *i.e.* compounds that can react and quench reactive oxygen species (ROS). In plants, this is possibly their most important function, either reacting with singlet oxygen ($^1O_2$), or acting as electron donors and acceptors for free radicals such as peroxyl (ROO·), hydroxyl (OH·) or alkoxyl (RO·) radicals. Usually, the longer the extended conjugated π-electron system that may stabilize the radical, the higher the antioxidant capacity.

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Carotenoid biosynthesis in plants is achieved *via* the condensation of isopentyl diphosphate (IPP) units, resulting in the formation of phytoene possessing nine double bonds, which is then further desaturated to lycopene (13 double bonds), following then further cyclization to α- or β-ionone rings, and/or hydroxylation processes, resulting in the formation of oxocarotenoids or xanthophylls such as lutein or zeaxanthin. Finally, expoxidation reactions can occur, with the formation of, for example, capsanthin, violaxanthin, neoxanthin, and fucoxanthin (Bohn 2008).

Carotenoids can also be further classified into provitamin A and non-provitamin A carotenoids. Although it is assumed that provitamin A carotenoids contribute only to a third of vitamin A supply in the typical Western diet, this proportion is possibly much higher in developing countries with low meat intake or for vegetarians. Among the provitamin A carotenoids that can be cleaved by human enzymes and transferred into retinol are β-

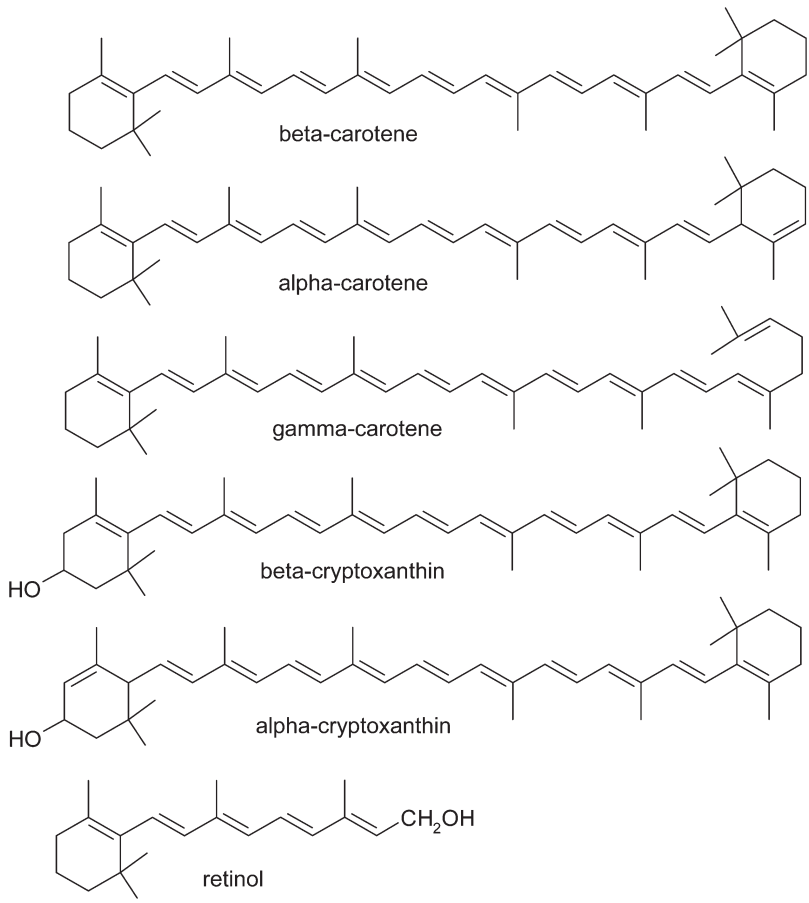

Figure 10.1 Carotenoids with provitamin A activity and retinol. Structures of provitamin A carotenoids frequently found in the diet.

carotene, α-carotene, γ-carotene, and α- and β-cryptoxanthin (Figure 10.1). While central enzymatic cleavage of β-carotene eventually results in the formation of two vitamin A active compounds, all other provitamin A carotenoids can only yield one vitamin A active molecule.

The biological activity of provitamin A carotenoids depends on many factors, including dietary and host-related factors. Provitamin A carotenoid absorption is usually low, in the area of 10–20% (Bohn 2008), and not all absorbed provitamin A carotenoids are finally cleaved by β-carotene 15,15′-mono-oxygenase (BCMO1) into vitamin A active compounds. Thus, the

Table 10.1 Major mechanisms for anticipated beneficial health effects of provitamin A carotenoids. Despite their main activities relying on their vitamin A activity and acting as antioxidants, a number of additional effects could contribute to their proclaimed health benefits. COX-2, cyclo-oxygenase 2; EGF endothelial growth factor; IGF, insulin-like growth factor; IL, interleukin; MDA, malondialdehyde; NF-κB, nuclear factor κB.

Effect	*Description*	*Reference*
Antioxidant	Protection of DNA against peroxidation and strand break	Bouayed and Bohn (2010)
	Prevention of lipid peroxidation	Bouayed and Bohn (2010)
	Reduction of MDA and other reactive products of peroxidation	Reifen *et al* (2004)
	Improving endothelial function *via* impact on NO formation	Fang *et al* (2009)
	Inhibition of LDL oxidation, thrombosis, platelet aggregation	Halliwell (2000)
Cell proliferation	Tumour suppression activity	Reddy *et al* (2005)
	Increased intracellular oxidative stress in tumor cells	Palozza *et al* (2004)
	EGF and IGF receptor protein down-regulation	
Apoptosis	Several pathways: - up-regulation of the anti-apoptotic Bcl-2 protein - increased mitochondria pore transition permeability - induced mitochondrial cytochrome *c* - activation of caspases - increasing NF-κB-binding sites and mediating apoptosis	Palozza *et al* (2004)
Anti-inflammatory	COX-2 down-regulation	Palozza *et al* (2004)
	Reduction of pro-inflammatory cytokines IL-1β and IL-6 mRNA in macrophages	Konishi *et al* (2008)
Cellular communication	Improved gap junction communication	Stahl and Sies (2001)

efficiency of cleavage of provitamin A carotenoids into vitamin A, also referred to as bioefficacy, has been the topic of many controversial discussions. Some organizations such as the Food and Agriculture Organization (FAO)/ World Health Organization (WHO) claim an activity of 1:6 compared with pure vitamin A, whereas others suggest 1:12 (Institute of Medicine 2001). Thus, given a dietary reference intake (DRI), *e.g.* the recommended dietary allowance (RDA) for vitamin A of 900 μg day^{-1} for an adult male (Institute of Medicine 2001), an intake of 11 mg of β-carotene would ensure sufficient vitamin A supply, assuming a conversion rate of 1:12. The average dietary β-carotene consumption is typically lower, approx. 5–10 mg, suggesting that additional vitamin A or other provitamin A carotenoids have to be consumed to meet the dietary recommendations for vitamin A intake.

However, the potential biological activity of provitamin A carotenoids, also including non-vitamin-A-related activities, have recently received increased attention due to epidemiological studies associating their consumption within fruits and vegetables with several beneficial health effects, especially for their potential to reduce the incidence of chronic diseases such as cancer and cardiovascular diseases (CVDs). Although often predominantly attributed to their antioxidant potential, carotenoids may further impact on gap junction communication between cells, regulate cell differentiation, growth and apoptosis, and modulate gene expression and interact with drug-metabolizing enzymes (Table 10.1).

10.2 Occurrence of Provitamin A Carotenoids in the Diet

In most native plant food items, both provitamin A carotenoids and non-provitamin A carotenoids occur concurrently. Due to their affiliation with the chlorophylls and the light-harvesting complex, and their presence in chromoplasts, coloured fruits and vegetables, especially leafy vegetables, are rich sources of carotenoids, up to approx. 10–15 mg $(100\ g)^{-1}$. Some root and tuber vegetables, such as carrots and sweet potato, respectively, also constitute provitamin A carotenoid-rich sources (Table 10.2). Also plant species residing in the sea, such as green algae, can be rich in provitamin A carotenoids, however, these sources are rarely consumed in Western countries.

Although animals cannot produce carotenoids, some species accumulate carotenoids in their tissues, such as some fish and seafood. For example, salmon is comparatively rich in the non- provitamin A astaxanthin. Eggs can also be a source of secondary carotenoids, especially lutein and zeaxanthin, originating from maize fodder, whereas their β-carotene content is very low (Table 10.2). However, in general, animal food products are poor sources of carotenoids. A number of foods are further fortified with carotenoids, especially β-carotene. Predominant examples include butter, dairy products, such as cheese, and a number of beverages. In addition to a potential prevention of oxidation and the formation of off-flavours or rancidity, such as in margarine, β-carotene is also added for colour preservation, resulting in a

Table 10.2 Dietary sources rich in carotenoids. List of common food items rich in carotenoids, listing both provitamin A carotenoids and total carotenoids. n.d., no data.

Source/food item	*Total carotenoids*[a] *[mg (100 g)$^{-1}$]*	*Provitamin A carotenoids*[b] *[mg (100 g)$^{-1}$]*	*Reference*
Carrots, raw	10.4	10.2	O'Neill *et al* (2001)
Sweet potato	n.d.	8.6	Max Rubner-Institut (2011)
Kale, raw	n.d.	5.2	Max Rubner-Institut (2011)
Salmon	n.d.	5	Max Rubner-Institut (2011)
Spinach, cooked	10.8	4.5	O'Neill *et al* (2001)
Grapefruit, pink	4.7	1.3	O'Neill *et al* (2001)
Apricot, fresh	1.1	1.0	O'Neill *et al* (2001)
Lettuce, butterhead	2.1	0.9	O'Neill *et al* (2001)
Chicken eggs	<0.1	0.6	O'Neill *et al* (2001)
Tomato, raw	3.4	0.6	O'Neill *et al* (2001)
Green bell-pepper, raw	n.d.	0.5	Max Rubner-Institut (2011)
Pumpkin, cooked	1.1	0.5	O'Neill *et al* (2001)
Butter	0.4	0.4	O'Neill *et al* (2001)
Green beans, cooked	1.1	0.4	O'Neill *et al* (2001)
Watermelon, peeled, ripe	3.7	0.2	O'Neill *et al* (2001)
Plum, raw, unpeeled	0.1	0.1	O'Neill *et al* (2001)
Sweet corn	0.9	0.1	O'Neill *et al* (2001)

[a]In edible portion. [b]Sum of α- and β-carotene.

yellow hue. Another source are dietary supplements, either in form of multimineral/multivitamin supplements, or as individual supplements, with typical doses of up to 15 mg of β-carotene unit^{-1}, most typically a capsule.

Of the carotenoids present in the diet, the predominant ones include β-carotene, lycopene, lutein, β-cryptoxanthin and α-carotene (O'Neill *et al* 2001). Some databases containing information on carotenoid content include the US carotenoid database (Mangels *et al* 1993), and a European database (O'Neill *et al* 2001), and more general databases such as the German Bundeslebensmittelschlüssel (Max Rubner-Institut 2011). It is interesting to note that many carotenoids have so far not been systematically included in these databases. This is especially true for a number of non-provitamin A carotenoids, such as phytoene or phytofluene, or expoxycarotenoids including violaxanthin and neoxanthin (Biehler *et al* 2011a).

It is further noteworthy that carotenoids are present in different forms in various food items. Although in many vegetables such as tomato and carrots, carotenoids are mostly present as agglomerates in crystalline form in the

chromoplasts and/or chloroplasts; carotenoids in some fruits such as oranges and watermelon exist dissolved in oil. These differences are of potential importance, as the presence in crystalline form could correspond with reduced release and solubilization from the food matrix and therefore reduced bioavailability. Another distinguishing form includes esters of hydroxycarotenoids and fatty acids, *i.e.* α- and β-cryptoxanthin esters. Some fruits and vegetables may contain the majority of β-cryptoxanthin in form of various esters (Breithaupt and Bamedi 2001). Due to their increased apolarity, these are also assumed to be of poorer bioavailability compared with the free form.

10.3 Dietary Intake of Provitamin A Carotenoids

In contrast to vitamin A, no dietary intake recommendations exist for provitamin A carotenoids. Nevertheless, with respect to the associated health benefits, dietary carotenoid consumption has been investigated in several countries. Two common ways to study dietary patterns include studying food disappearance, which is based usually on sales data and suffers from inaccuracy due to the fact that not all purchased food is eventually consumed by humans; or, preferably, food consumption studies where data from individual subjects are compiled and averaged. However, even these estimates are easily biased due to: (1) source of data with respect to carotenoid content of food items, (2) the population studied, *e.g.* rural *vs.* metropolitan, (3) assessment methods such as 24 h recall or diet history, and (4) neglecting certain dietary sources such as supplements.

Despite these difficulties, a number of studies have aimed at assessing carotenoid intake, showing that average carotenoid consumption is in the region of 10–20 mg day^{-1}, and approx. 30–50% of this is usually attributed to provitamin A carotenoids (O'Neill *et al* 2001). The majority of the carotenoids consumed are in form of α- plus β-carotene, followed by lycopene, lutein and β-cryptoxanthin. Similar as for the food databases, consumption of carotenoid precursors (phytoene, phytofluene) and several minor abundant carotenoids, such as the expoxycarotenoids, are usually, but not always (Biehler *et al* 2011a) neglected, albeit they could contribute to approx. 25% of total carotenoid intake.

Despite differences between countries in their carotenoid consumption, with, for example, northern countries typically consuming less carotenoids than, for example, Mediterranean countries, carotenoid intake depends on an individual's diet. For example, vegetarians consume more fruits and vegetables than omnivorous people, which was found to translate into approx. 25% higher plasma β-carotene levels (Johnson *et al* 1995).

10.4 Detection of Provitamin A Carotenoids in Food Items and Body Tissues

Following extraction methods, typically employing liquid/liquid extraction such as with hexane/acetone or clean-up by solid phase extraction (SPE), the

detection of carotenoids including provitamin A carotenoids most often relies on their strong ultraviolet–visible (UV-Vis) activity, making use of their specific, often three-finger-type absorption bands between 450–500 nm. However, absorption wavelengths could differ, with shorter wavelengths for carotenoids with a less extended conjugated electron system such as phytoene, absorbing in the area of approx. 285 nm. A reduction of the conjugated π-electron system also results in decreased absorption sensitivity, typically expressed by their specific absorption coefficients (Table 10.3).

The easiest quantification of carotenoids is *via* simple spectrophotometric methods. However, carotenoids mostly occur in mixtures, and their detection can be further impeded by the presence of other spectrophotometric active compounds such as chlorophylls. If the aim is to only detect the sum of carotenoids, chlorophylls could be degraded *via*, for example, saponification, and total carotenoid content can be estimated based on the mean of their absorption coefficients (Biehler *et al* 2009). Another method is to subtract chlorophylls mathematically *via* measuring at several wavelengths to distinguish between chlorophylls and carotenoids (Lichtenthaler 1987). However, individual carotenoids cannot be determined.

Thus, prior to their UV-Vis detection, carotenoids are usually separated chromatographically. Although carotenoids are too fragile to be determined by gas chromatography, high-pressure liquid chromatography (HPLC) is able to allow for individual separation. Often as extracts from plants are analyzed which could contain residues of water, reversed phase (RP) chromatography has become the method of choice for carotenoid detection. Due to their long chain length, in addition to the standard RP-18 configuration, special carotenoid columns with RP-30 materials have been developed, which even allow the separation of various isomers, such as of 9-*cis* and all-*trans* β-

Table 10.3 Specific molecular absorption coefficients of carotenoids. Selected, dietary predominant carotenoids, their specific molecular absorption coefficients and their maximum absorption wavelengths in the UV-Vis area.

Carotenoid	*Solvent*	*Absorption maxima (nm)*	*Specific molecular absorption coefficient [L mol^{-1} cm^{-1}]*	*Reference*
β-Carotene	Acetone	452	140663	Britton *et al* (2004)
Lutein	Ethanol	445	144900	Britton *et al* (2004)
Lycopene	Acetone	448	120600	Britton *et al* (2004)
Zeaxanthin	Acetone	452	133118	Britton *et al* (2004)
β-Cryptoxanthin	Petrol Ether	449	131915	Britton *et al* (2004)
α-Carotene	Hexane	445	145472	Britton *et al* (2004)
Phytoene	Hexane	286	73567	Campbell *et al* (2007)
Phytofluene	Hexane	348	67863	Campbell *et al* (2007)

carotene, and also allow for detecting different lycopene isomers (Hadley *et al* 2003). However, to date, no ultra-pressure/performance liquid chromatography (UPLC) column with this material exists. When employing RP-30 HPLC, the time for a complete carotenoid profile detection is approx. 25–40 min. The detector of choice coupled to HPLC analysis is the diode array detector (DAD), allowing the detection of each individual cartotenoid at its maximum sensitive wavelength.

In addition to UV-Vis-based methods, two other techniques are worth mentioning: electrochemical detection (ECD) and mass spectrometry (MS), both of which are usually coupled to HPLC. ECD oxidizes carotenoids at specifically applied electrochemical potentials, thus another dimension is added for separating carotenoids in addition to retention time. ECD equipment, such as with the Coularray detector (ESA, Chelmsford, MA, USA), has been claimed to be 10–1000 times more sensitive compared with UV-Vis detectors, albeit due to the high UV-Vis activity of carotenoids, the gain in detection is possibly on the lower side. However, several methods have reported on the use of ECD for carotenoid analyses (Bohn *et al* 2011; Ferruzzi *et al* 1998), with detection limits as low as 0.1 ng mL^{-1} for standards. This may be the method of choice for studying carotenoid breakdown products or carotenoids with shorter conjugated π-electron systems and reduced UV-Vis activity.

MS is possibly the priciest choice to detect carotenoids. Both HPLC-MS and HPLC-tandem MS (MS-MS) methods, especially in combination with atmospheric pressure chemical ionization (APCI) have been suggested, with the latter technique reaching sensitivities of 0.1 ng mL^{-1} in human plasma (Gundersen *et al* 2007).

10.5 Aspects of Bioavailability of Provitamin A Carotenoids

10.5.1 Overview of Provitamin A Carotenoid Absorption

Factors that impact on provitamin A carotenoids have been summarized by the mnemonic term SLAMENGHI: species of carotenoids, molecular linkage, amount of carotenoids ingested, food matrix effects, dietary factors effecting absorption and bioconversion, nutrient status of the host, genetic factors of the host, host factors such as intestinal passage time, and the interaction of all these factors. The absorption of carotenoids can be thought of as the following sequence (Failla and Chitchumroonchokchai 2005) (see Figure 10.2):

1. Release from food matrix
2. Solubilization and micellarization for cellular uptake
3. Uptake into the epithelial cells of the small intestine
4. Transport through the epithelial cells, partly central cleavage, reaction to retinal, then retinol and re-esterification to, for example, retinyl palmitate

5. Sequestration for further absorption into chylomicrons and transport *via* lymph to the bloodstream
6. Remodelling in the liver and further transport in various lipoprotein fractions
7. Transport to various organs, incorporation into, for example, lipid bilayers
8. Excretion *via* urine and feces, following breakdown into smaller apo-carotenals and unknown compounds.

Thus, all factors impacting any of these processes can affect the bioavailability of provitamin A carotenoids (Table 10.4). In the following, the above stages are outlined in further detail.

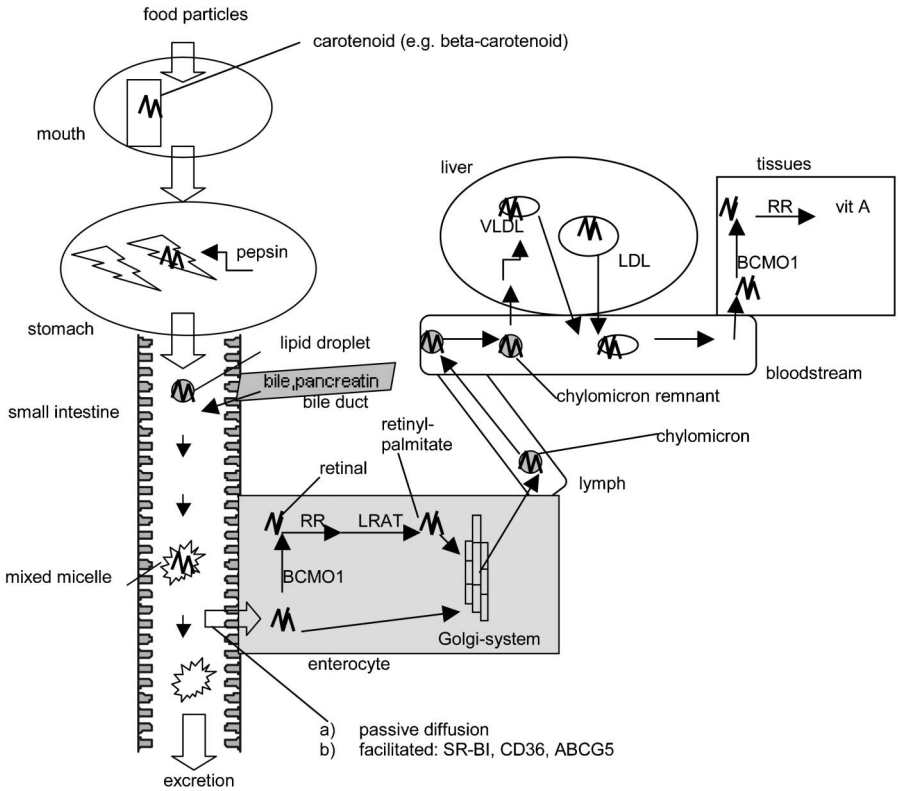

Figure 10.2 Various stages of biodistribution of provitamin A carotenoids following ingestion *via* the diet. The Figure shows carotenoid release from the matrix, micellarization, cellular uptake, cleavage into retinol and re-esterification, sequestration into chylomicrons, remodelling in the liver and further transport in lipoproteins and tissue uptake. RR, retinal reductase; vitA, vitamin A; LRAT, lecithin:retinol acyltransferase; SR-BI, scavenger receptor class B type I; ABCG5, ATB-binding cassette G5; BCMO1, beta-carotene 15,15′-mono.oxygenase 1.

10.5.1.1 Release from Food Matrix

Following dietary intake, carotenoids have first to be released from the food matrix by chewing and enzymatic digestion. The more intact the plant cell matrix, the harder the plant material and cell walls, and the more viscous the food item during gastro-intestinal digestion, the less available the carotenoid fraction is for further uptake. In addition, carotenoids in larger, more crystalline-like agglomerations, such as in the chloroplasts of leafy vegetables, or in chromoplasts, such as in tomato or carrots, could be hypothesized to be less soluble compared with carotenoids present in lipid droplets, such as in orange and watermelon.

Processing, especially the heating of fruits and vegetables, has shown to considerably improve carotenoid absorption, especially that of lycopene, in humans and animals, most likely due to maceration of the plant cell walls, destruction of the crystalline agglomerates or release of the protein-bound carotenoids. However, heating has also been reported to foster *trans–cis* isomerization of carotenoids, *i.e.* formation of 9-*cis* β-carotene from all-*trans* β-carotene. While this form has been reported to undergo micellarization to a larger extent compared with all-*trans* β-carotene, it also appeared to be of lower cellular uptake (Canene-Adams and Erdman 2009). Carotenoids in supplements are usually extracted from carotenoid-rich plants, such as spinach, and can be assumed to be at least equally well released and absorbed compared with dietary sources.

10.5.1.2 Solubilization/Micellarization for Cellular Uptake

Due to their low solubility in water, carotenoids have to undergo micellarization, *i.e.* emulsification prior to their uptake by the epithelium in the small intestine. This micellarization usually requires the presence of some dietary-derived lipids such as triacylglycerols and phospholipids which are later broken down enzymatically, such as into free fatty acids and mono- and diglycerides. During digestion, carotenoids are first present in lipid droplets, which then, during gastro-intestinal digestion, and together with free fatty acids, phospholipids, cholesterol and the bile salts, result in the formation of mixed micelles of approx. 8 nm size (Parker 1996). The more polar carotenoids, such as xanthophylls including β-cryptoxanthin, are located in the outer layers of this micelle, whereas the more apolar ones, such as β-carotene, are believed to be located in the core (Borel *et al* 1996). Due to their improved water solubility, the more polar carotenoids typically show higher micellarization (Biehler *et al* 2011b). The same has been found for the *cis*-forms as opposed to the normally present all-*trans*-forms, perhaps due to lower apparent chain length and easier incorporation into mixed micelles (Ferruzzi *et al* 2006).

A number of factors have been reported to interact with the formation of mixed micelles. Several studies have shown that the consumption of dietary lipids together with a carotenoid-rich meal improves the availability of carotenoids, presumably due to improved micellarization (Biehler *et al* 2011b;

Unlu *et al* 2005). A negative impact on the micellarization process has been suggested by compounds competing with emulsification, such as higher amounts of other carotenoids, *e.g.* lutein, or other lipid-soluble vitamins such as vitamin E, vitamin D or phytosterols, albeit there exists no conclusive data (reviewed by Bohn 2008). Furthermore, a lack of digestion enzymes, such as pancreatin, and bile acids would result in impaired micellarization (Biehler *et al* 2011b). Recently, it has been suggested that high concentrations of divalent minerals could reduce the availability of fatty acids and bile salts in the gut, due to the formation of insoluble soaps, also reducing carotenoid micellarization (Biehler *et al* 2011b).

10.5.1.3 Uptake into the Epithelial Cells of the Intestine

Uptake into the epithelial cells of the small intestine is believed to occur following the diffusion of the micelles through the unstirred water layer to the apical site of the mucosal cells. Earlier, it was believed that cellular uptake is based on solely passive diffusion. In recent years, however, based on Caco-2 cellular trials, it was suggested that also facilitated uptake mechanisms for several carotenoids, such as *via* the scavenging receptor type B class I (SR-BI), do play a role. This protein is normally responsible for cholesterol uptake, and blocking this receptor (with ezetimibe) also inhibited uptake of carotenoids, especially of β-carotene and α-carotene, but to a lesser extent that of lycopene and lutein (During *et al* 2005). The fact that β-carotene uptake has been shown to be saturable (Failla and Chitchumroonchokchai 2005) is in further agreement with this assumption. It therefore appears likely that the uptake of the more essential provitamin A carotenoids is additionally regulated at this stage of absorption. Other transport proteins, including cluster determinant 36 (CD36) and the ATB-binding cassette G5 (ABCG5), have also been assumed to play a role during carotenoid uptake, albeit their role remains to be elucidated further.

10.5.1.4 Transport through the Epithelial Cells, Central Cleavage and Re-Esterification

Following their uptake into the enterocytes, provitamin A carotenoids may then be cleaved by central enzymatic cleavage, by the enzyme BCMO1, possibly in the cytosol. Retinal is then further reduced by retinal reductase into retinol which is then esterified by lecithin:retinol acyltransferase (LRAT) with C-16 or C-18 fatty acids such as palmitate for further transport. Also, an asymmetric or eccentric cleavage by, for example, β-carotene 9′,10′-dioxygenase (BCDO2) may occur (Lindqvist *et al* 2005), resulting in the formation of different apo-carotenals, compounds that could still possess vitamin A activity, as chain reduction and retinal formation could still occur. Both enzymes appear to be active in a variety of epithelial cells, including the parenchymal cells of the liver, the adrenal gland, kidney tubules, among

others. Also non-enzymatic oxidation of carotenoids to apo-carotenals could occur, but this is unlikely to be the major pathway of biotransformation, as other animal species, such as the cat, lack the ability to produce significant amounts of vitamin A from β-carotene. 9-*cis* β-Carotene, which may be formed during food processing or gastro-intestinal digestion, is believed to be isomerized into its *trans*-form within the enterocytes, either prior to or after cleavage, as their plasma levels are very low.

Based on human studies with isotopically labelled β-carotene, it has been estimated that 35–71% of absorbed β-carotene can be cleaved into vitamin A active compounds (van Vliet *et al* 1995), albeit lower conversions of approx. 20% have also been discussed (reviewed by Parker 1996). Factors affecting the intracellular cleavage process are only poorly understood, but it can be speculated that re-esterification could depend on the presence of recently absorbed dietary-derived fatty acids.

10.5.1.5 Sequestration for Further Absorption into Chylomicrons and Transport in Lymph and Blood

The non-cleaved carotenoids and the re-esterified retinyl palmitate are then sequestered *via* the Golgi apparatus of the enterocyte into chylomicrons, which then reach via the lymphatic system and the thoracic duct the bloodstream, where they are transformed under the cleaving influence of lipoprotein lipase into chylomicron remnants. As only newly absorbed cartotenoids can be found in this fraction, this is a preferred analytical target to study carotenoid absorption by investigating the so-called triacylglycerol-rich lipoprotein fraction (TRL), mostly consisting of chylomicrons (Parker 1996), especially its area under a curve (AUC) (concentration *vs.* time).

The sequestration of chylomicrons by the Golgi apparatus is assumed to be impacted on by following test meals containing dietary lipids, as carotenoid absorption following a single carotenoid meal is usually biphasic, with a second peak in the plasma-TRL fraction following a carotenoid-free but fat-containing lunch (Unlu *et al* 2005). The additional lipids are assumed to foster the sequestration of additional chylomicrons into the lymph and therefore facilitate carotenoid absorption.

10.5.1.6 Remodelling in the Liver and Further Transport in Lipoproteins

In the liver, chylomicron remnants are endocytosed by parenchymal hepatocytes and may be stored or packaged into various lipoproteins, considered to be the only transport vehicle of carotenoids in the bloodstream. While the more apolar carotenes are transported preferably by very-low-density lipoproteins (VLDLs) and low-density lipoproteins (LDLs), approx. 10–16% and 58–73%, respectively, the dihydroxy-xanthophylls are rather associated with high-density lipoproteins (HDLs; approx. 53%, *vs.* 31% and 16% in LDLs

Table 10.4 Factors impacting aspects of provitamin A carotenoid bioavailability. The impact of positive and negative host and external factors on provitamin A carotenoid availability at various steps of biodistribution. LCFAs, long-chain fatty acids; N/A, not available; TGs, triacylglycerols; ROS, reactive oxygen species; SR-B1, scavenger receptor class B type 1.

Stage of biodistribution	*Factor influencing bioavailability*	*Positive/negative effect*	*Literature*
Digestion, release	Dietary fibre	−	Failla and Chitchumroonchokchai (2005)
	Viscous matrix	−	Biehler *et al* (2011b)
	Heat processing of food	+	Unlu *et al* (2007)
Micellarization	Competing compounds (*e.g.* other carotenoids)	−	Bohn (2008)
	Minerals	−	Biehler *et al* (2011b)
	Dietary lipids	+	Unlu *et al* (2005)
	Presence as carotenoid ester	−	Bohn (2008)
	Lack of digestion enzymes or bile salts	−	Biehler *et al* (2011b)
Cellular uptake	Drugs blocking SR-B1 (*e.g.* ezetemibe)	−	During *et al* (2005)
	High viscosity of chyme	−	Failla and Chitchumroonchokchai (2005)
Cleavage into vitamin A	Presence of vitamin A in diet	−	Failla and Chitchumroonchokchai (2005)
Chylomicron sequestration	Following test meals containing TGs composed of LCFAs	+	Bohn *et al* (2011)
Biodistribution	Unknown	N/A	N/A
Metabolism/ excretion	Presence of ROS, UV-Vis damage of skin	+	Speculative

and VLDLs, respectively). β-Cryptoxanthin was found to equally partition between LDLs and HDLs, approx. 40% each (Furr 2004). Overall, LDLs are believed to account for >50% of total carotenoid transport in the human body (Canene-Adams and Erdman 2009). Peak appearance of β-carotene in LDLs were found approx. 24–48 h following dietary intake (reviewed by Parker 1996), which is much delayed compared with the plasma-TRL fraction, where carotenoids usually peak at approx. 4–6 h following absorption.

In general, the most prevalent carotenoids found in plasma are lutein and lycopene, followed by β-carotene and ζ-carotene, β-cryptoxanthin and α-carotene (Khachik 2006), which together possibly contribute to approx. 90% of the carotenoids in the human body (Rao and Rao 2007). However, the profile of carotenoids in plasma can vary largely and is thought to reflect the general dietary patterns. On carotenoid-depleted diets, significant reductions in carotenoid blood levels by over 50% were detected, with half-lives between 26–76 days. The normal average concentrations of carotenoids in blood plasma are in the range of approx. 0.3–0.7 μmol L (Canene-Adams and Erdman 2009).

10.5.1.7 Target Organs in the Human Body

There exists no predominant single target organ for provitamin A carotenoids, therefore, these can be found in all tissues. Instead, there are several organs with increased affinity, depending presumably on the number of LDL receptors and SR-BIs. Adipose tissue, prostate, liver, testis and adrenal tissue possess a high expression of LDL receptors (reviewed by Biehler and Bohn 2010) and therefore constitute preferential targets for provitamin A carotenoids. Adipose tissue may thus constitute a useful marker of long-term carotenoid intake (Canene-Adams and Erdman 2009). In addition, comparatively high concentrations of β-carotene and degradation products were found also in lung, breast and colonic tissue (Khachik *et al* 2002). A number of carotenoids including β-carotene can also be detected in the skin where they could offer protection from UV-induced damage. In addition, the liver remains the major storage tissue for provitamin A carotenoids, and returning lipoproteins including their carotenoids could be remodelled in this organ. In all organs, additional provitamin A carotenoid conversion into vitamin A can continue.

10.5.1.8 Aspects of Excretion of Provitamin A Carotenoids and Vitamin A

Not much is known on the further biological fate of provitamin A carotenoids following uptake by body tissue cells. In lipid bilayers, provitamin A carotenoids have been reported to be present in the core of the phospholipid membrane. This is in contrast to the xanthophylls which reside, due to increased hydrophilicity, more in the outer parts (Parker 1996). Thus, a high fraction of carotenoids is usually affiliated with the membrane fraction of cells, protecting these from ROS and lipid peroxidation.

Excretion of non-absorbed provitamin A caroteoids occurs mostly *via* the feces, whereas absorbed provitamin A carotenoids are partly excreted *via* the bile or pancreas into the feces. Interestingly, large amounts of isotopically labelled β-carotene were recovered in the urine (approx. 35% of those absorbed) (Lemke *et al* 2003), possibly following breakdown *via* shorter apo-carotenals and their hydroxylated products.

Summary Points

- This Chapter focuses on provitamin A carotenoids.
- The main sources of provitamin A carotenoids include leafy vegetables and coloured fruits, containing up to approx. 15 mg/100 g of provitamin A carotenoids.
- The main dietary provitamin A source is β-carotene, followed by β-cryptoxanthin and α-carotene.
- Predominant detection and quantification methods include spectrophotometry (UV-Vis) and HPLC, especially reversed phase HPLC coupled to a diode array detector.
- Bioavailability is low, mainly due to low bioaccessibility and limited cellular uptake.
- Health aspects include not only vitamin A aspects, but also anti-inflammatory, anti-proliferative and antioxidant effects.

Key Facts

Key Facts of General Properties of Provitamin A Carotenoids

- Among the primary function of provitamin A carotenoids is their central cleavage in various cells of the human body by 15,15′-monooxygenase and their subsequent transformation into retinoic acid.
- β-Carotene has the highest provitamin A activity of all carotenoids, as its central cleavage will eventually produce two vitamin A molecules. A key feature of possessing vitamin A activity is the intact β-ionone ring.
- α-Carotene, γ-carotene, α-cryptoxanthin and β-cryptoxanthin can result in only one vitamin A molecule following central cleavage.
- The bioefficacy, *i.e.* the efficiency of provitamin A carotenoids to result in the formation of vitamin A in the human body, is controversial, and estimates include both a biological activity of 1:6 and 1:12 compared with vitamin A.
- In addition to their potential vitamin A activity, provitamin A carotenoids have, especially in *in vitro* and animal studies, further shown to possess anti-inflammatory, anti-proliferative and pro-apoptotic properties. Their interaction with gene expression has also been highlighted.

Key Facts of Dietary Intake and Bioavailability of Provitamin A Carotenoids

- Rich dietary sources are leafy vegetables and some coloured fruits. Dietary carotenoids are either bound to proteins, *i.e.* to the light-harvesting complex

in the chloroplasts of plants, occur in rather crystalline form within chromoplasts, appear dissolved in lipid droplets as in some fruits.

- The general dietary intake of provitamin A carotenoids is approx. 5–10 mg day^{-1}, which may not be enough to result by itself in sufficient vitamin A supply to meet the recommended dietary intake (approx. 0.9 mg day^{-1}).
- Even if high amounts of provitamin A carotenoids are consumed, they are typically of low bioaccessibility and absorption. Co-ingested dietary lipids can enhance their absorption, whereas dietary fibre will possibly reduce bioavailability.
- Earlier thought to occur by passive diffusion, cellular uptake of provitamin A carotenoids could also take place *via* facilitated uptake *via* proteins (e.g. SR-BI).
- Absorption of newly consumed carotenoids in the body can be followed by examining the plasma chylomicron fraction, while plasma and especially LDL-affiliated carotenoids represent the major circulating fraction.

Definitions of Words and Terms

Antioxidant capacity: The potential of a nutrient or phytochemical to act as an antioxidant and to prevent the formation or negative activities of ROS.

Bioaccessibility: The amount of a nutrient or phytochemical that can be released from the food matrix, solubilized and be available for further uptake and absorption.

Bioavailability: The fraction of a nutrient or phytochemical that can be absorbed and is available for its physiological functions and/or storage.

Bioefficacy: In this Chapter, the efficiency or extent to which provitamin A carotenoids are cleaved by central cleavage and result eventually in the formation of vitamin A.

DRI-RDA: The dietary reference intake (DRI) of the US Food and Nutrition Board is an umbrella term for intake recommendations of certain nutrients, especially vitamins and minerals. The recommended dietary allowance (RDA) is the intake that supplies the majority of the population (>97%) with a sufficient amount to ensure optional body health and functions.

Phytochemical: A dietary compound with no strict essentiality for humans, but which may have nevertheless an impact on the human body. Examples include polyphenols and carotenoids.

Provitamin A carotenoids: Carotenoids that can result, following enzymatic central cleavage in human cells, in the formation of vitamin A. Only carotenoids with an intact β-ionone ring possess provitamin A activity.

Provitamin A carotenoid status: A measure of the overall supply of the human body with provitamin A carotenoids. The best measure of a status of a nutrient is the measurement of its concentration in the primary target organ. As such a target tissue does not exist for carotenoids, measurements usually include blood plasma or adipose tissue.

Reactive oxygen species (ROS): These can be classified into radicals and non-radicals, but both types have the potential to result in cellular damage, such as of the lipid bilayer membrane, or could result in protein and DNA damage. Examples include the hydroxyl radical ($OH^{\cdot}$) or hydrogen peroxide (H_2O_2).
Uptake: Transport of a nutrient or phytochemical from an extracellular space (*e.g.* the intestinal lumen) through the cellular membrane (*e.g.* of the enterocyte) into the cytosol of the cell.

List of Abbreviations

ABCG5	ATB-binding cassette G5
BCMO1	β-carotene 15,15′-mono-oxygenase 1
CD36	cluster determinant 36
ECD	electrochemical detection
HDL	high-density lipoprotein
HPLC	high-pressure liquid chromatography
LDL	low-density lipoprotein
LRAT	lecithin:retinol acyltransferase
MS	mass spectrometry
ROS	reactive oxygen species
RP	reversed phase
RR	retinol reductase
SR-BI	scavenger receptor class B type I
TRL	triacylglycerol-rich lipoprotein fraction
UV-Vis	ultraviolet or visible light
VLDL	very-low-density lipoprotein

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